EXPOSURE SYSTEM FOR SMALL ANIMALS AT ATMOSPHERIC AND REDUCED PRESSURES

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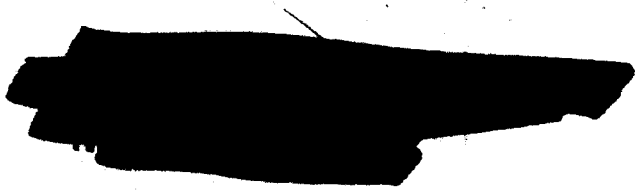
Running Head: Atmospheric and Reduced Pressure Exposure System

ABSTRACT

An exposure system is described which provides for chronic exposure of experimental animals (rats) to selected gaseous environments of varied composition and pressure (150-760 mm Hg absolute). The system includes specially designed exposure capsules, a gas flow system, an automatic pressure regulation system, and a respiratory gas analyzer for operation at both atmospheric and reduced pressures. The system has been operated at reduced pressure (450 mm Hg absolute) for a period of 64 days with no apparent operational problems and provided ±5 mm Hg pressure control. Air control animals at atmospheric pressure over this period demonstrated that: (1) the capsule environment did not restrict the animals, and (2) growth rates and food consumption data did not differ appreciably from that for animals in metabolic cages.

INDEX TERMS

exposure chambers, ambient exposure system, reduced pressure exposure system, respiratory gas analysis



INTRODUCTION

The gas atmosphere for human subjects in a spacecraft may differ from air in composition and may not be at atmospheric pressure. A great deal of research has been performed on environmental control systems and habitation studies, but a notable information gap still exists concerning the toxicological aspects. In order to help fill this gap, the Ames Research Center is studying toxicological aspects of artificial atmospheres. This research will include studies of various oxygen concentrations, mixed gases, and trace contaminants associated with the current concepts of the closed spacecraft ecological system.

In order to perform these studies, it was necessary to design special chamber equipment and instrumentation for small animals. This paper describes a system which provides for chronic exposure of animals (rats) to selected gaseous environments of varied composition and pressure (150-760 mm Hg absolute) without the inherent complexities and cost of large chamber operation. This report is subdivided into four areas: (1) construction, (2) operation, (3) system discussion, and (4) experimental results.

CONSTRUCTION

Exposure capsules.- The individual exposure capsules, constructed of clear acrylic plastic, allow continuous observation of the experimental animals (see Fig. 1). The capsules have a free volume of approximately 4.2 liters. Of this volume 2.8 liters is available for animal occupancy. A fine-mesh stainless steel screen rests on a raised section in the center of the capsule base. The center tube of a gas distribution ring fits through the screen and is connected to the gas inlet fitting of the base. A coarse

Fig. 1

galvanized wire screen then fits into a groove in the base above the gas distribution ring. The coarse screen allows the fecal material to drop through onto the stainless screen, and urine flows through to a reservoir in the base, thus providing separation of excreta.

The domes of the capsules include a spring loaded, solid feeder mechanism, a drinking tube and cap assembly, a flat plastic plate on top of the raised dome sections, and a transfer tunnel and valve. The feeder mechanism holds approximately 35 g of food pellets, and the drinking tube holds approximately 120 ml of water. The flat plate on top of the capsule dome contains the exhaust gas fitting for the capsule and a hermetically sealed electrical connector with an attached thermistor probe for monitoring air temperature. The transfer valve is a gate valve designed to seal the capsule during chronic exposures. When opened, it allows the animal to crawl into a clean chamber for uninterrupted exposure.

To insure against gas leaks, O-ring seals are used between all clamped surfaces of the capsule assembly and under the feeder and water tube caps. Silicone stopcock grease is used on the O-rings.

Gas supply.- For toxicity studies with gases such as oxygen, a supply of tanked gas is sufficient. When mixed gases are to be used, however, supplies of the desired gas mixtures or the pure gases and appropriate gas mixing equipment can be employed. The gas supply for this exposure system includes oxygen (from a liquid oxygen source - 99.8% pure) and water pumped compressed air. Because of the cost and logistics problem of obtaining specific gas mixtures in large quantities over a prolonged time period, four gas mix tanks[1] have been installed to provide a controlled mixture of gases to the exposure capsules. For other pure gas sources, a storage area has

been provided, and a special low pressure manifolding and piping system has been installed to connect these sources to the appropriate gas mix tanks.

Gas flow system.- The components of the exposure assembly are illustrated in Figs. 2 and 3. Metered gas enters a capsule through the base. The capsule exhaust passes through the normally open port of a three-way solenoid valve, through a needle valve, and into an exhaust manifold common to all capsules. A gas flow line from the water tube cap is also connected to this manifold. Each capsule has its own entry and exhaust flow systems, including the gas metering system and the valves in the exhaust line. An absolute pressure gauge is used to monitor the pressure in the exhaust manifold. The manifold is connected to a surge tank by three flow paths. One path contains a ball valve, and each of the other two paths contains a vacuum regulator in series with a toggle valve. The surge tank is connected to the two vacuum pumps and to ambient (atmospheric pressure) through swing check valves. (Two 1-1/2-hp vacuum pumps each with a capacity of 30 SCFM (Standard Cubic Feet per Minute) are installed as the vacuum source.)

Figs. 2, 3

Gas analyzer.- The surge tank is also connected to a gas analyzer by way of a vacuum regulator in series with a toggle valve. The analyzer is portable and can be connected to the normally closed port of the three-way solenoid valve of any selected capsule exhaust system. The analyzer includes a carbon dioxide analyzer,[2] an oxygen analyzer,[3] a dew point hygrometer,[4] appropriate valving, flow and pressure indicators, and recording equipment (see Figs. 3 and 4).

Fig. 4

OPERATION

Reduced pressure.- For operation below atmospheric pressure, the exhaust manifold is adjusted to the desired pressure. The experimental animals are

placed in the capsules, and the ventilating gas flow is connected. When the gas composition differs from air, the capsules are purged at a high ventilatory flow rate (1.5 liters/min) for 10 min. The exhaust lines are connected and the capsules are evacuated to the desired pressure level. During this procedure, it is essential to maintain the gas pressure in the drinking tubes below that in the capsules. Otherwise, the water will not be retained in the drinking tubes. Also, the rate of change of pressure in the capsules must be regulated for the safety of the animals. The gas flow rates can be adjusted to control CO_2 or water vapor in the capsules at desired levels.

In order to maintain the gaseous environment free from metabolic contaminants and to provide adequate food and water, the experimental animals must be transferred to clean capsules daily. The transfer tunnels of the "change" capsules are clamped to the transfer valves of the "on-line" capsules, and the change capsules are purged and evacuated to the pressure of the on-line capsule. The animals are transferred through the valves to the clean capsules (see Fig. 5). The dirty capsules are then disconnected and washed. This transfer mechanism makes it possible to maintain continuous uniform exposure for extended periods.

Fig. 5

Atmosphere pressure.- If the capsule environment is to remain at atmospheric pressure, the capsule exhaust is connected to a separate exhaust manifold at atmospheric pressure. The purge procedures will depend upon the composition of the ventilating gas.

Weight determinations.- Animal weight changes that occur during an exposure period may be required during the experimentation. The most difficult weighting task is to determine the weight changes of animals in capsules at reduced pressures. To accomplish this, a special capsule is equipped with separate valving that allows the capsule to be completely isolated from the gas flow

and vacuum system and the on-line capsule. The animal is transferred from the on-line capsule to the weighing capsule, which has been equilibrated to the same pressure and composition. The animal and capsule are weighed together, and the animal is transferred back to the original capsule. The total procedure takes approximately 2 min. Weighing animals in a gaseous capsule environment other than air, but at atmospheric pressure, requires essentially the same procedure, with the exception of the exhaust pressure controls.

Gas analyses.- To determine the levels of respiratory gases in the capsules, the following procedure is used: The analyzer inlet is connected to the closed solenoid port of the selected capsule exhaust and the detectors are equilibrated to the proper pressure. The solenoid is energized and the capsule exhaust gas is diverted through the analyzer rather than the exhaust manifold. To discontinue the exhaust flow through the analyzer, the solenoid is de-energized. The analyzer can then be connected to another capsule exhaust system.

SYSTEM DISCUSSION

Exposure system.- As stated previously, this exposure system is designed to provide a facility for conducting toxicity studies over the range of absolute pressures from 150-760 mm Hg. For reduced pressure exposures, the surge tank is maintained at an absolute pressure between 25 and 100 mm Hg (dependent on total flow rate into the system), and the vacuum regulators maintain the exhaust manifold at the desired absolute pressure level. (The regulators have small orifices; therefore two regulators are installed in parallel pathways to insure an adequate flow rate and pressure control of the exhaust manifold.)

The individual capsules are sealed except for the inlet and exhaust gas flows, and the restrictions through the capsule to the exhaust manifold are minimal. The capsules are, therefore, maintained at essentially the same absolute pressure as the mainfold +2-3 mm Hg.

One of the vacuum pumps is used as the on-line pump and the other as an emergency pump. A pressure switch, mounted in the surge tank, activates the emergency pump in the event of failure of the on-line pump. The pumps are alternated for routine maintenance while exposure studies are in progress. An emergency power system is being installed to guard against the loss of electrical power.

The surge tank and the swing check valves have been installed to prevent sudden recompression of the exposure capsules. If a loss in the electrical power and/or vacuum occurs, the swing check valve between the pump and the surge tank closes. The reduced pressure in the tank maintains the capsules at their respective pressures for a short period of time and allows them to recompress to atmospheric pressure slowly. In case of vacuum failure, the rate of recompression is dependent on the number of capsules connected to the exhaust system. For example, if 14 capsules are being maintained at 400 mm Hg absolute pressure with a ventilatory gas flow of 800 ml/min per capsule, the exhaust manifold will be maintained at 400 mm for approximately 15 min. The tank, the exhaust manifold, and the capsules will then begin to pressurize and will reach atmospheric pressure in approximately 15 min. When the complete exhaust system exceeds atmospheric pressure by 15 mm Hg, the ambient swing check valve on the surge tank opens and allows the exhaust to bleed out, thus preventing pressurization of the exposure capsules.

As mentioned previously, this exposure system was designed for experimental animals the size of rats. With slight modifications, the capsules could be used for larger animal species, if desired.

Gas analyzer.- During exposure periods, it is extremely desirable to monitor levels of respiratory gases in the capsules. It is also desirable to perform such analyses at the capsule pressure with a high degree of accuracy. Previous attempts were made by the authors to use repressurization techniques and Scholander's (1) analyses of capsule exhausts. The procedure proved to be time consuming and inadequate. Even if appropriate gas detection equipment is available, the best of the repressurization techniques creates data analysis problems. Such techniques cause additional mixing of a gas sample and render a continuous monitoring task difficult.

In order to circumvent these problems, the gas detection system was designed to monitor capsule exhaust at the pressure (atmospheric or reduced) of the capsules. The resistance to gas flow in the analyzer is greater than in the capsule exhaust line; therefore to maintain the capsule pressure constant, the analyzer is evacuated to a slightly lower pressure than the exhaust manifold. The limiting accuracy of the analyzer is as follows: oxygen ±1 mm PO_2, carbon dioxide ±0.05%, water vapor ±0.3 mm P_{H_2O}.

EXPERIMENTAL RESULTS

These capsules were designed to study gaseous composition and pressure effects on experimental animals. Although control animals are routinely employed, it is desirable to know if confinement in the capsules imposes growth and metabolic restrictions on the animals.

To determine this, studies were made with rats in the exposure capsules at atmospheric pressure with compressed air as the ventilating gas. Three

groups of 7 male rats were maintained in the air capsule environment for 10, 28, and 64 days, respectively. Three control groups were also maintained in metabolic cages in an air environment at atmospheric pressure. The capsule groups did not demonstrate any significant differences in weight changes when compared to the control groups. The growth curves of the experimental animals for the 64-day experiment are illustrated in Fig. 6. The food consumption data were subjected to statistical analysis, and the data for the three capsule groups did not differ significantly from that for the animals in the metabolic cages.

Fig. 6

Additional studies were also carried out with groups of rats exposed to oxygen in the same type capsule at various absolute pressures (250, 450, 600 mm Hg). Throughout these exposures, the system operated successfully and provided pressure control of ±5 mm Hg. One of these groups was maintained at 450 mm for 64 days. The results of this exposure are reported by G. A. Brooksby and R. W. Staley of Ames Research Center and will appear in a separate publication.

ACKNOWLEDGEMENTS

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REFERENCE

1. Scholander, P. F. Analyzer for accurate estimation of respiratory gases in one-half cubic centimeter samples. J. Biol. Chem. 167: 235-250, 1947.

FOOTNOTES

[1]Linde RC-2A gas ratio controllers.

[2]Mine Safety Appliance Company Model 200 Lira infrared analyzer.

[3]Beckman Instrument Company Model F-3 oxygen analyzer (paramagnetic).

[4]Cambridge Systems Model 990 thermoelectric dew point hygrometer.

FIGURE LEGENDS

Fig. 1.- Capsule for chronic exposure (exploded view).

Fig. 2.- Exposure capsules on station. (The flow and pressure control panels are included.)

Fig. 3.- Gas flow diagram:

V_1 - needle valves

V_2 - toggle valves

V_3 - ball valves

V_4 - swing check valves

Fig. 4.- Gas analyzer.

Fig. 5.- Experimental animal transferring to clean capsule.

Fig. 6.- Growth curves for male rats in exposure capsules and metabolic cages. (The gaseous environment is air at atmospheric pressure for both groups of animals.)

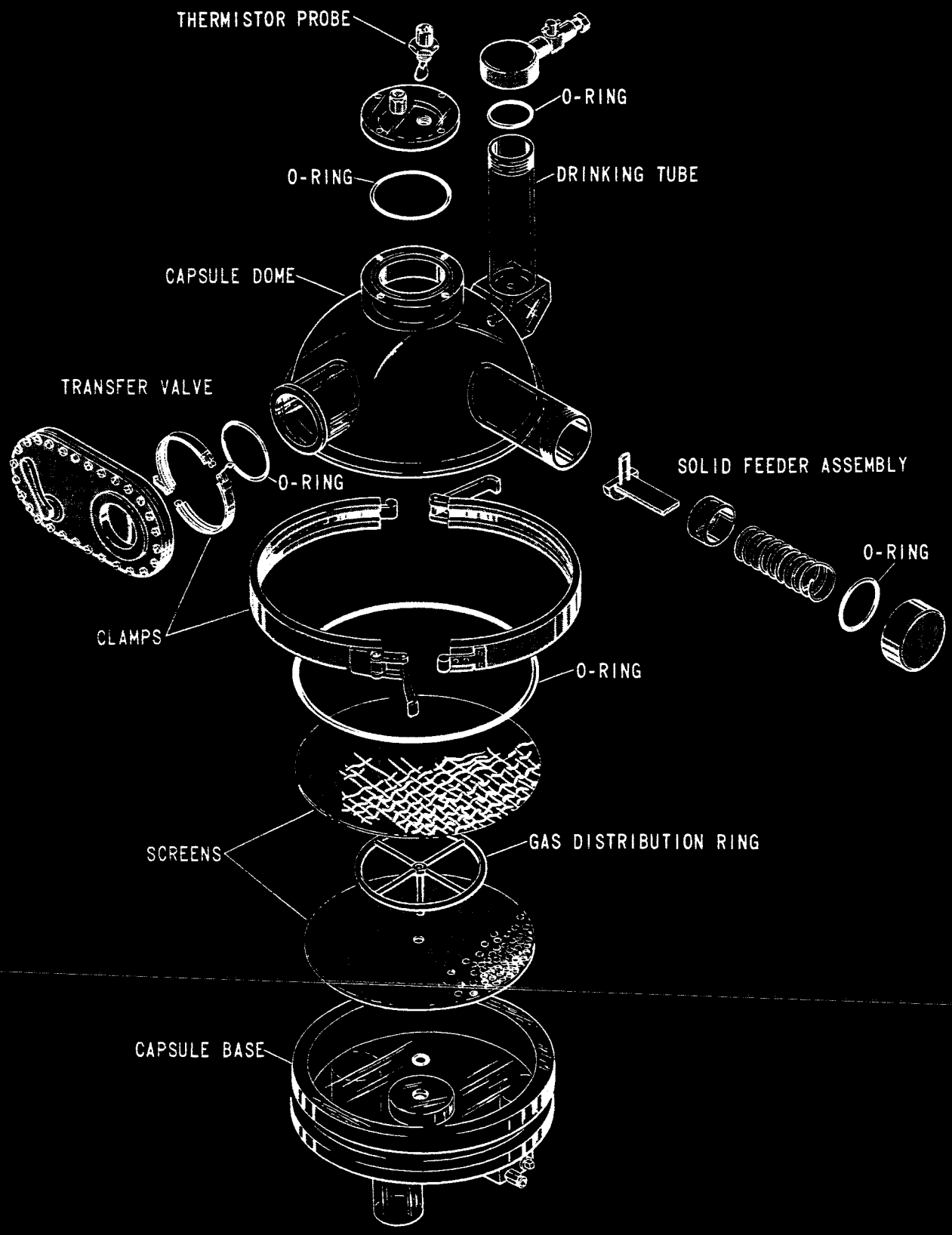

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Fig. 1

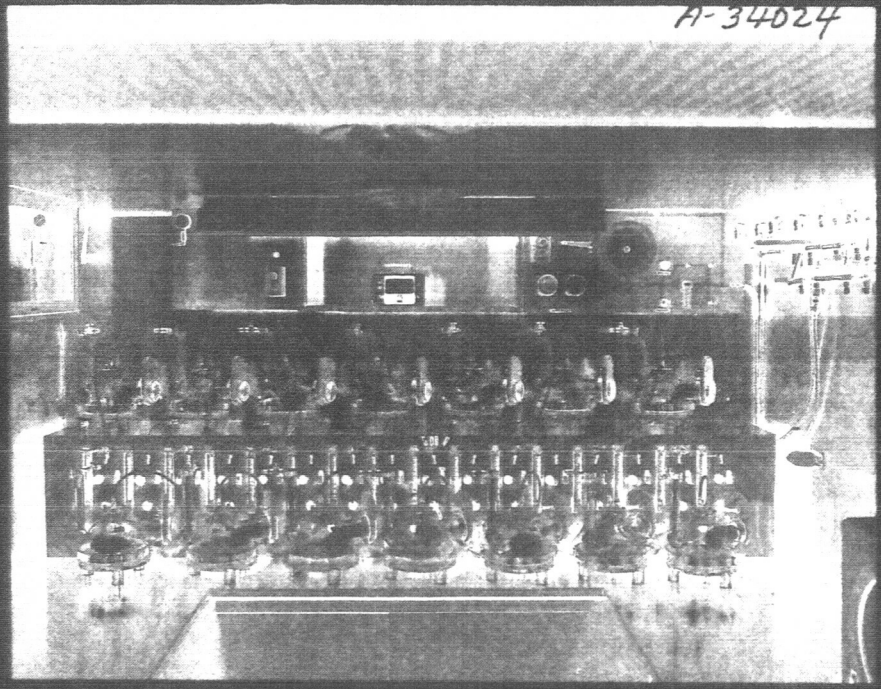

Fig. 2

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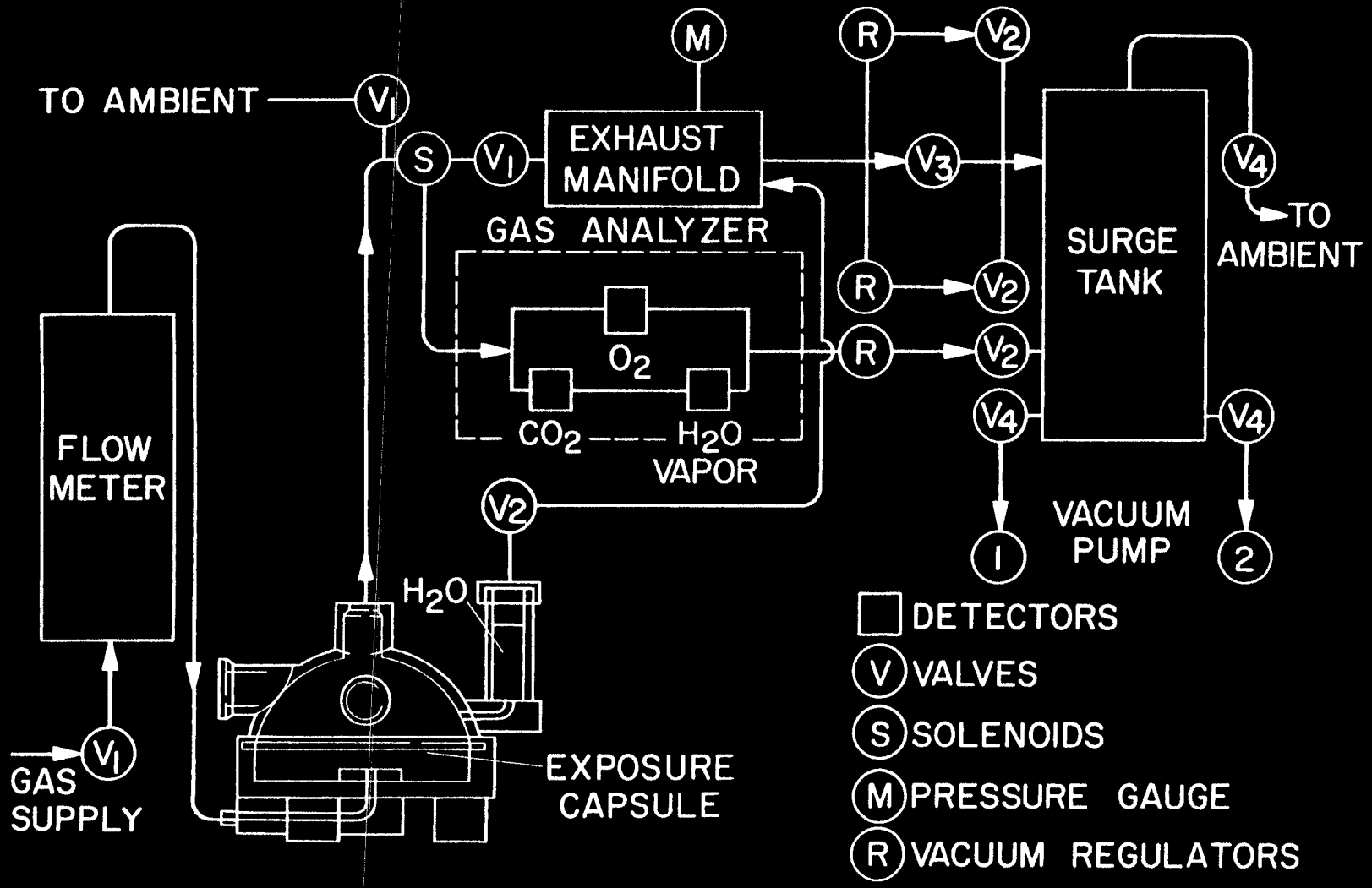

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Fig. 3

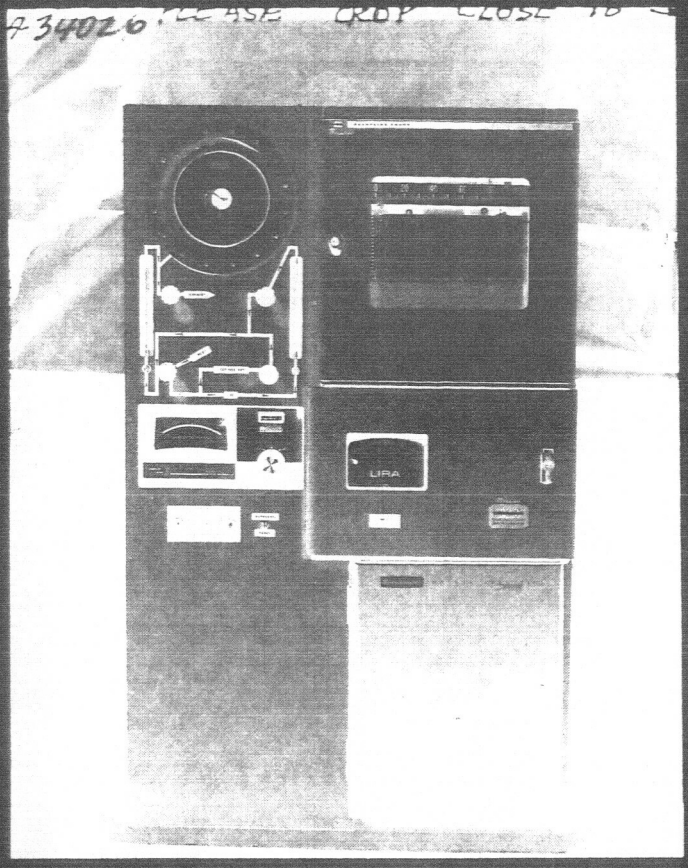

Fig. 4

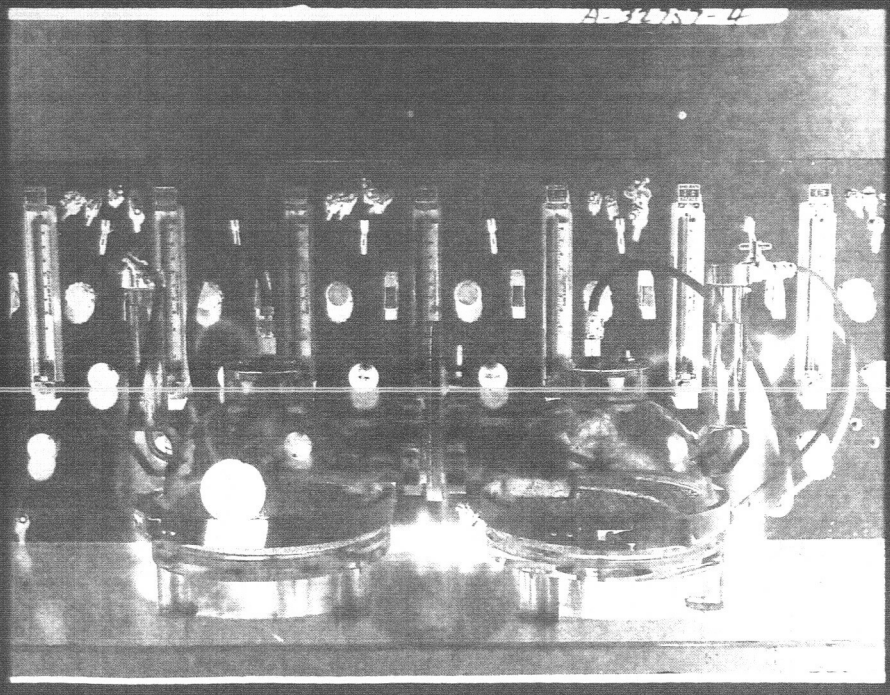

Fig. 5

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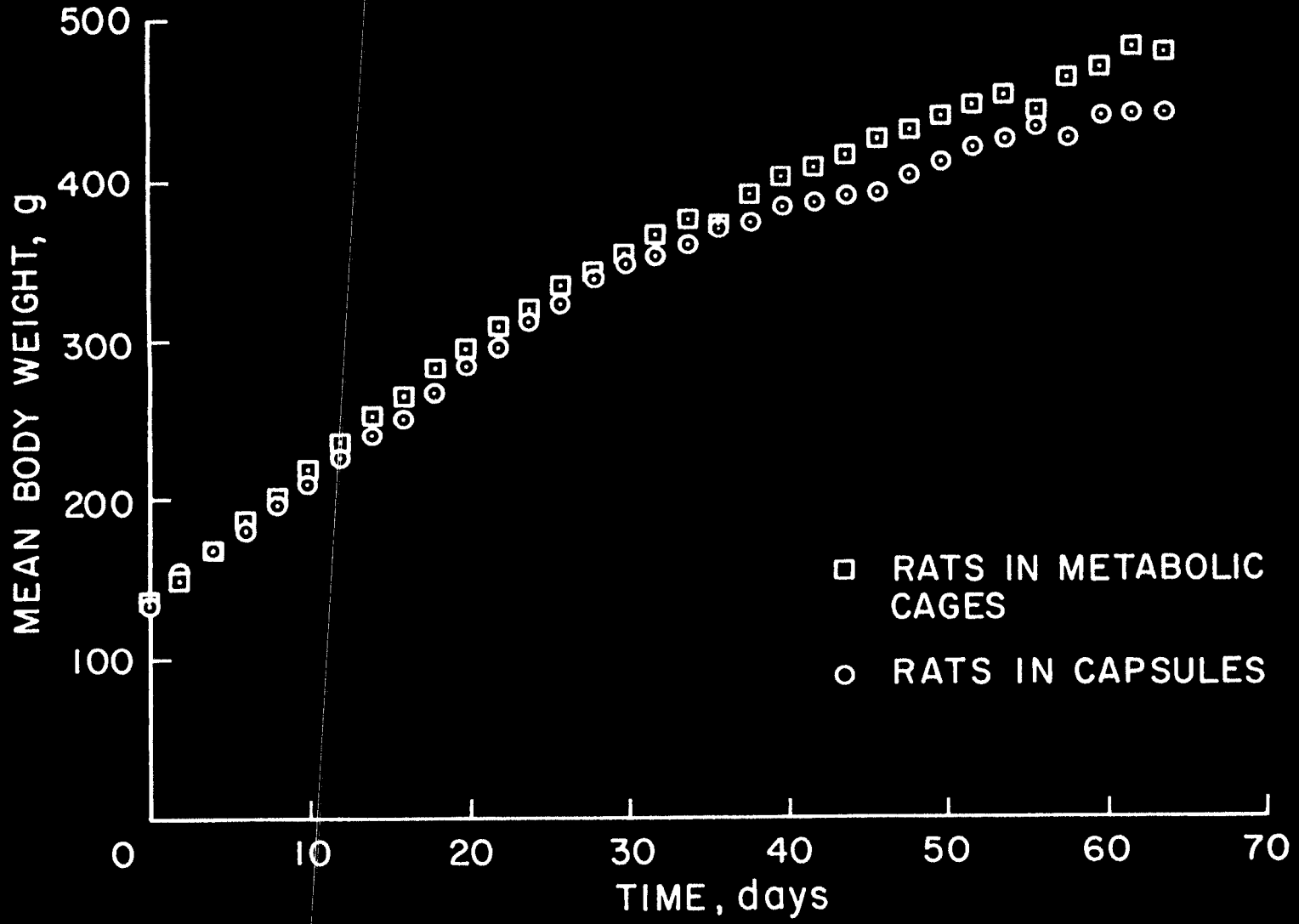

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Fig. 6